Innovations

p53 in 3-D

Since its discovery in 1979 and its more recent recognition as a significant cancer gene, p53 has eluded researchers seeking to construct a three-dimensional image of the protein it encodes. Recently, a team of scientists headed by Nikola P. Pavletich at the Sloan Kettering Memorial Hospital in New York has achieved this goal through X-ray crystallography and computer modeling of the p53 protein's DNA-binding portion—where most of its dangerous mutations occur. The availability of this structural information provides "a framework for understanding how mutations may inactivate the p53 protein," said Pavletich. This discovery may lead to the design of drugs that reverse its cancer-causing defects.

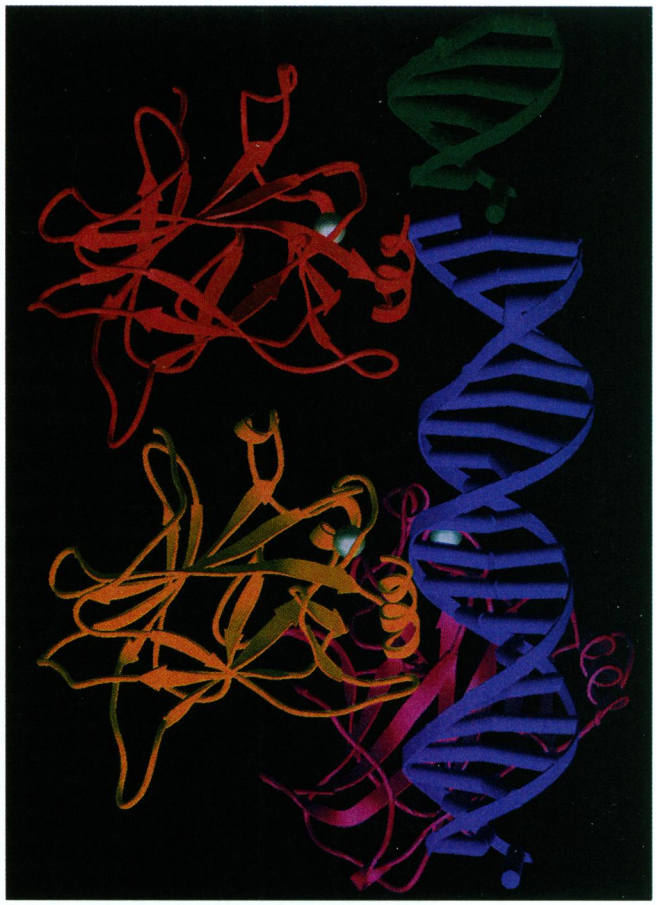
Role of p53

Known as the "guardian of the genome," and named 1993 "Molecule of the Year" by *Science,* p53 protects human DNA. The p53 gene encodes for a tumor-suppressor protein that provides surveillance and direction of many cellular activities. Normal p53 is active in control of the cell cycle, determining when and if cells are replicated through interactions with various genes and proteins. When a piece of DNA is damaged, p53 detects the problems and stops DNA replication, allowing repair of the site. The p53 protein is also thought to play a role in apoptosis, an orderly cell suicide that occurs when a cell receives a signal to self-destruct. Also called programmed cell death, apoptosis is a normal process during growth and development, helping the body to rid itself of injured or obsolete cells. When p53 is mutated, it is unable to perform these vital functions, thereby allowing cancerous cells to survive and proliferate. In fact, altered p53 proteins are found in more than 50% of all cancers.

Located on the short arm of human chromosome 17, the p53 gene is subject to mutations at almost every point of its gene sequence. Most of its mutations are missense mutations, in which one DNA base is exchanged for another. Specific environmental chemicals can cause perilous changes in the p53 gene. Exposure to aflatoxin B_1, a food toxin endemic to areas of Asia and Africa, causes transversions of a particular guanosine to thymidine in the p53 sequence. Guanosine-to-thymidine changes are often found in cases of hepatic cancer. Cigarette smoke causes similar transversions throughout the p53 gene that are found in lung and head and neck cancers. Ultraviolet light, on the other hand, causes alterations in the chemical bonding of cytosines and thymidines. Base-pair changes are seen in colon and brain cancers, as well as in lymphomas and leukemias.

While most p53 mutations occur in somatic (body) cells and therefore only induce disease in the individuals carrying the defective gene, mutations in germline cells (egg and sperm) affect succeeding generations. An example of this is Li-Fraumeni syndrome (LFS), a mutation of p53 associated with soft-tissue sarcomas and osteosarcomas. Researchers are finding that individuals who inherit LFS are subject to a number of different cancers from a young age. Patients with a p53 mutation have a 50% chance of having cancer before the age of 30 and a 90% chance before the age of 65. Mutations of p53 may create genetic instability, making individuals susceptible to other types of genetic diseases.

The results of molecular genetics studies have led to hopes of improved methods of cancer diagnosis, prognosis, and gene-based treatments. In the future, genetic screening may become a routine part of a trip to the doctor's office. Finding a defective tumor-suppressor gene before symptoms are evident would allow increased monitoring and early detection of disease, which would be especially important for families affected by LFS, for example. The presence of a mutated p53 appears to be an important gauge of prognosis, since cancer patients with aberrant p53 genes have lower survival rates and poorer response to treatment than patients with normal p53 genes. In addition to increasing the accuracy and speed of diagnosis, genetic screening strategies may be applied in trials of drugs designed to prevent cancer and assist in choosing which chemotherapy or radiation regimens should be used.



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Crystal clear. Nikola P. Pavletich uses X-ray crystallography to create a 3-D image of the p53 protein.

Replacing a defective p53 gene through gene therapy may provide the ideal solution for individuals with mutations. Although p53 gene therapy clinical trials are in progress for patients with lung cancer at the M.D. Anderson Cancer Center in Houston, a number of technical problems still remain. Delivering the healthy p53 genes to the right cells and at the right place in the genetic material, getting the newly inserted healthy genes to produce the protein and to regulate it properly, and making sure that insertion of the new gene and its subsequent expression do not cause new problems are all hurdles that face gene therapy.

Crystal Challenges

Elucidating p53's three-dimensional structure offered many challenges, particularly in the science of X-ray crystallography. Successful crystallography has three essential components: availability of a pure protein sample, production of a crystal of the protein, and the technology to collect and analyze X-ray diffraction data. For several decades, scientists have been able to manipu-

late the DNA of microorganisms to quickly synthesize large amounts of protein macromolecules. Such efficient and rapid production obviates the traditional painstaking isolation and purification procedures that often resulted in low quantities of protein that were marred by contaminants. Although molecular advances have solved the problem of sufficient quantity and purity, the rate-limiting step—growing the crystals—is still a difficult task.

In X-ray crystallography, a crystal, a tightly packed, highly organized structure of identical, repeating copies of a molecule, is bombarded with beams of X-rays on one side at varying angles. These beams are recorded onto film on the other side of the crystal. Depending on whether the beam encounters particles (atoms) or empty spaces in the structure, it scatters to form characteristic patterns on the film. These patterns are measured and, using complex mathematical equations, are used to construct the locations and arrangements of atoms within the molecule.

X-ray beams bounce off flat, uniform surfaces in a measurable and predictable manner. To visualize proteins, which have irregular, intricately folded shapes, the proteins must be in the form of a crystal. The basic structure of a crystal, called the unit cell, holds a certain number of copies of the protein under study. Geometry dictates that there are only six possible shapes for unit cells. This makes the number of protein copies within a unit cell dependent on a variety of factors, including the type, size, electrical charges, and arrangement of its amino acids. A typical procedure used to grow crystals is to supersaturate an organic solvent with the protein and to dehydrate the solution with a crystallizing chemical agent that forces the protein molecules to build lattice structures. If the chemical agent is added slowly enough to the protein solution, crystals form instead of an unorganized residue.

The natural properties of proteins makes their crystallization more tedious than crystallization of salts like sodium chloride. Biological molecules, including proteins, form fewer bonds with their neighbors than inorganic chemicals because of their large size, making protein crystals relatively more fragile. Proteins are literally forced together by the chemical environment when forming crystals, so the bonds that are created are quite weak. Because proteins are very sensitive to changes in temperature or solvent conditions, they can easily lose their natural shape and become gel-like instead of solid when crystallized. "Growing crystals is not cut and dry," says Alexander McPherson, a crystallographer at the University of California, Riverside. But improvements in every aspect of the process have caused "a burst in the number of new protein structures," he adds.

Surprises and Satisfaction

The third component of X-ray crystallography, collection and analysis of X-ray data, has also taken a major leap forward since its early days. X-ray crystallographers and structural biologists have been able to "solve" (determine the 3-D structure of) a number of biological molecules since the early 1900s. In 1913 William Bragg worked out the initial mathematical equations by which scientists determine the structure of molecules in a crystal after bombardment by X-rays. Much of these mathematics were done by hand, with pencil and paper. Similar X-ray techniques and calculations used by James Watson and Francis Crick led to the discovery of DNA's double helix structure in 1953. A few years later, Max Perutz and John Kendrew applied X-ray crystallography to outline the structures of hemoglobin and myoglobin, the first proteins to be solved with X-ray crystallography. For many decades, collecting and analyzing X-ray data "was like trying to catch one fish at a time," said McPherson.

In more recent years, crystallographers have teamed up with computer modelers to formulate graphics programs that assemble 3-D pictures of proteins and DNA fragments with the input of X-ray data. Computer graphics also allow researchers to simulate interactions of a protein with other biochemical molecules, the possible twists and turns a protein might endure when placed in certain chemical environments, and the effects of removing or changing parts of its structure. These developments, along with improvements in X-ray-generating equipment and detection technology, have greatly accelerated the pace with which scientists can determine protein shapes. "Now, new structures appear at the rate of one per day" said McPherson.

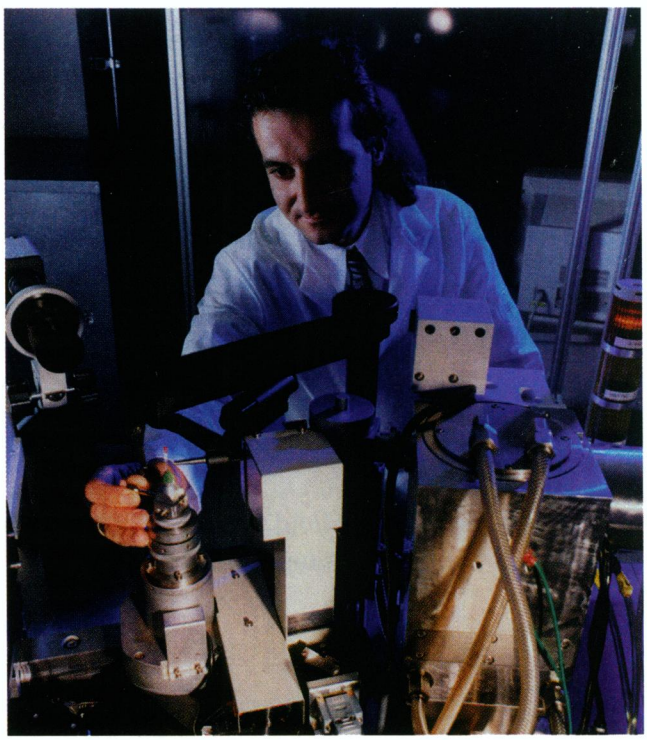
The analysis of Pavletich's crystal revealed a unit cell that contained three copies of p53 protein's central region or "core domain" attached to a DNA fragment. The solution disclosed both expected characteristics as well as some surprises. Unanticipated was the uniqueness of the protein; the p53 core domain does not resemble structurally any other DNA-binding region known to date. The amino acids of the p53 core domain fold into a particularly large shape, which is unusual among proteins that interact with DNA. The protein also displays more predictable aspects of a DNA-binding protein in that it makes typical contacts along the edges of the DNA helix.

One of most important results from this work is that the p53 gene's six "hot spots," areas of the gene that are most frequently mutated in cancer, correspond to specific amino acids that are altered in the protein's core domain, said Curtis Harris at the National Cancer Institute in Bethesda, Maryland. This set of mutations apparently reduces the overall stability of the p53 protein–DNA complex by altering p53's structure, thus abolishing the essential contacts made by the protein with DNA to suppress the formation of tumors.

Molecular Medicine

Now that the protein's structure is available, scientists "have a framework for understanding how mutations may inactivate the p53 protein," said Pavletich. Such framework may lead to procedures to reverse the progress of cancer.

One of the most promising applications of this work is in drug development. "We hope that by using the new information about p53's structure, we may learn how to design drugs that restore activity to mutated p53 protein, enabling it to perform its tumor-suppressing duties," Pavletich said. With the aid of computer graphics, researchers can use the 3-D image to begin creating compounds that combine with the p53 protein. Pharmacologists and medicinal



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Pictures of the puzzle. Computer modeling of the DNA-binding portion of p53 will help scientists understand how mutations occur in this protein.

chemists will most likely streamline their approach to seeking drugs that will correct altered p53; instead of testing potential drugs at random for their ability to bind and repair the protein, knowledge of the structure may enable some drugs to be eliminated before commencing extensive testing.

Design of a curative drug is not simple or straightforward. "The 3-D structure will greatly facilitate us in designing a drug because it allows us to focus onto a particular region of the protein," says Jeff Ives of Pfizer, Inc., "but the job ahead is quite formidable." One reason for this is the tremendous diversity of p53 mutants. Reestablishing normal p53 function will mean forcing the protein back into its original conformation so that it can once again make its customary contacts. In some cases, this will involve making drugs that block aberrant interactions between large proteins. To correct the effects of other mutations, like those directly in the DNA-binding area, a new drug may have to function by obstructing the protein from regions adjacent to the core domain. It is expected that numerous types of drug's will be needed to correct all mutant effects in all types of cancer.

Although drug developers have found ways to correct defective enzymes and make changes in small specific receptor sites for certain molecules, genetic proteins represent one of their biggest hurdles. "Whether or not rational drug design can provide broad-based cancer therapeutics remains to be seen," said Ives. "Restoration of . . . a virtually denatured p53 molecule will be a Herculean task for any small [drug] molecule," Stephen Friend of the Massachusetts General Hospital Cancer Center in Boston wrote in a recent *Science* article.

Equally important to cancer research is the opportunity that scientists now have to further understand the function of p53. Studying a protein's structure to elucidate its function is a standard research approach. And with the increasing possibilities for finding cancer treatments, excitement in protein crystallography is expected to continue growing. "Different mutant forms of p53 have different bioactivities. Knowledge of the structure gives increased insight into how various mutations alter normal activity," said Harris. McPherson agrees: "Getting to the structure magnifies molecular biological studies. In the old days, there were probably fewer than 50 researchers worldwide trying to grow crystals; now, there is enormous interest in structural studies."

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Suggested Reading

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